Quantitative Comparison of Stylet Penetration Behaviors of Glassy-Winged Sharpshooter on Selected Hosts

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ABSTRACT New Zealand is threatened by invasion of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), an important vector of *Xylella fastidiosa*, a gram-negative bacterium that causes Pierce's disease in grape (*Vitis* spp.) and scorch diseases in many other horticultural crops. Therefore, an understanding of the host acceptability, feeding behavior, and potential vector efficiency of glassy-winged sharpshooter on New Zealand crops is important. We tested host plant acceptance and feeding behaviors of glassy-winged sharpshooter on three common horticultural crops grown in New Zealand (apple [*Malus* spp.], grape, and citrus [*Citrus* spp.]), and a native plant (*Metrosideros excelsa* [=*tomentosa*] Richard, pohutukawa), using the electrical penetration graph (EPG) technique. Probing (stylet penetration) behaviors varied among the host plants, primarily due to differences in waveform event durations. Apple and grape were the most accepted host plants, on which glassy-winged sharpshooter spent the majority of its time on the plant probing and readily located and accepted a xylem cell for ingestion. This resulted in long durations of sustained xylem fluid ingestion. In contrast, pohutukawa was the least accepted host. On this plant, glassy-winged sharpshooter spent less time probing and engaged in longer and more frequent testing/searching and xylem-testing activities, rejected xylem cells frequently, and spent less time with stylets resting, before accepting a xylem cell and ultimately performing the same amount of sustained ingestion. Citrus plants contaminated with sublethal insecticide residues were intermediate between these extremes, with some acceptance of xylem, but less ingestion, probably due to presumed partial paralysis of the cibarial muscles. Implications of the results in terms of host plant acceptance and the development of a stylet penetration index are discussed.

KEY WORDS *Homalodisca coagulata*, electrical penetration graph (EPG), probing, host plant acceptance

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), formerly *H. coagulata* (Say) (Takiya et al. 2006), is a highly invasive, economically important vector of the bacterium *Xylella fastidiosa.* Glassy-winged sharpshooter transmits strains of the bacterium that cause Pierce's disease (PD) in grape, and several leaf scorches in plants, such as oleander and almond (Redak et al. 2004). Transmission of different strains of *X. fastidiosa* can cause other diseases in avocados, citrus, peaches, apricot, cherries, and many other fruit crops and ornamentals. Glassy-winged sharpshooter was introduced into California and first recorded in the southern part of the state in 1989 (Sorensen and Gill 1996). Since then, this insect has continued to spread northward in California, where it can be found on >100 plant species (Hoddle 2003). Glassy-winged sharpshooter has the potential to seriously disrupt the state's agricultural economy and nearly 1 million jobs that are related to agriculture. A study released in 2006 (Freeze 2007) showed that the total annual economic value of California's winegrape industry is estimated at US$51.8 billion. The total cost of research and management for PD/glassy-winged sharpshooter in California, from 1999 to 2004 (most recent data), has been >$166 million, with nearly $39 million of that spent in research (CDFA 2007).

In 1999, glassy-winged sharpshooter occurred in Tahiti, the cultural, political, and economic capital of French Polynesia, located in the southern Pacific Ocean. Abundance of glassy-winged sharpshooter in Tahiti became extremely high, with an average of 170 nymphs being caught in 1 min of sweep net sampling (Petit et al. 2008). Although no xylella diseases were reported, the large population of glassy-winged sharpshooter present on the island became a major nuisance to the human population and also a threat to endangered spider and other arthropod species (Grandgirard et al. 2008) in the Tahitian ecosystem. The glassy-winged sharpshooter population in Tahiti has been significantly reduced by a classical biological control program launched in 2004 (Petit et al. 2008). A similar biological control program has been established in

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California; however, some geographical areas of California where glassy-winged sharpshooter has become established have insufficient degree-day accumulation to fully support the parasitoid, reducing the program's effectiveness (Pilkington and Hoddle 2007).

From Tahiti, glassy-winged sharpshooter spread to neighboring islands, to Mo'orea by 2002 and to the Cook Islands (2,615 km northeast of New Zealand) in 2007. It is expected to continue its dispersal westward across the Pacific. Consequently, New Zealand is threatened by the potential arrival of the glassy-winged sharpshooter. Climex models indicate that climatic conditions exist for glassy-winged sharpshooter to establish throughout much of New Zealand (Hoddle 2004), and the potential for success of a biological control program is unknown.

There is no record of *X. fastidiosa* present in New Zealand. If it became established after introduction of glassy-winged sharpshooter into New Zealand, the cost to horticultural crops could be large. The horticultural industry in New Zealand has grown significantly in the past two decades, with exports rising from $481.2 million in 1985 to $2.36 billion in 2006. In addition, domestic sales of horticultural products were estimated at $2.5 billion, to make the total industry revenue of $4.8 billion in 2006 (HortResearch–The Horticulture & Food Research Institute of New Zealand Ltd. 2006). Thus, threats to New Zealand from glassy-winged sharpshooter and *X. fastidiosa* have been focused on the New Zealand wine grape industry and other horticulture industries such as apple (*Malus* spp.). Yet, glassy-winged sharpshooter also poses economic threats to native flora and fauna of New Zealand, and to the environment. For example, glassy-winged sharpshooter feeds on New Zealand native trees and shrubs (e.g., *Metrosideros*, *Pittosporum*, and *Olearia* spp.) planted as ornamentals in both California and Tahiti. Pohutukawa, *Metrosideros excelsa* (=*tomentosa*) Richard, an iconic native plant known as the "New Zealand Christmas tree," is a popular ornamental in southern California and other parts of the world. Considering the potential impact of glassy-winged sharpshooter and *X. fastidiosa* on New Zealand's horticultural industry and the environment, New Zealand cannot afford to ignore a problem of such potential magnitude.

One of the most sustainable, long-term management tactics for glassy-winged sharpshooter and *X. fastidiosa* in the future will be host plant resistance. Several efforts are underway in California to develop resistant grape (Walker and Riaz 2007). If horticultural crops important to New Zealand are included in current California research on glassy-winged sharpshooter feeding, transmission of *X. fastidiosa*, and development of host plant resistance, then the knowledge generated will benefit New Zealand horticulture if this pest becomes established there. Part of the California effort is the development of a stylet penetration index (SPI), a type of host plant resistance index that relies upon data from electrical penetration graph (EPG) studies.

The EPG technique is the most rigorous means of assessing feeding and host acceptance by hemipterans. EPG allows quantification of stylet penetration (probing) behaviors (Tjallingii 1985, Backus and Hunter 1989, Almeida and Backus 2004, Backus et al. 2005a), identification of behaviors related to plant pathogen transmission (Tjallingii and Prado 2001), and investigation of plant resistance mechanisms (van Helden and Tjallingii 2000, Sandanayaka et al. 2003). Histology of probed plant tissues to identify the location of salivary sheaths, stylet tips, or both in the plant, coupled with EPG, provides a waveform correlation with plant cell types penetrated (Tjallingii and Hogen Esch 1993). Backus et al. (2005a) identified and categorized stereotypical waveforms produced by *H. vitripennis* during stylet penetration on grape, and they correlated those waveforms with salivary sheath branch terminations in plant cells.

The main objective of this study was to use EPG to provide the first quantitative comparison among several host plants of stylet penetration behaviors and ease of xylem acceptance by adult glassy-winged sharpshooter. A previous EPG study (Almeida and Backus 2004) used similar quantitative methods, but it did not compare among different host plants. Three horticultural crop plants that are economically important to New Zealand, (grape, citrus, and apple) and one native ornamental plant (pohutukawa), were compared using alternating current (AC) EPG monitoring. An accurate assessment of the ease of stylet penetration, degree of xylem acceptance, and ingestion duration on these host plants was carried out by analyzing EPG waveforms that already had been characterized and defined from adult glassy-winged sharpshooter feeding on susceptible grape petioles (Almeida and Backus 2004, Backus et al. 2005a). These data are the first of several from ongoing studies that are being assembled for future meta-analysis for development of a glassy-winged sharpshooter stylet penetration index.

Materials and Methods

Insects and Plants. Glassy-winged sharpshooter adults were field-collected from ornamental shrubs in Bakersfield, CA. Insects were transported to the USDA quarantine facility in Fresno, CA (under USDA–APHIS permits nos. 2293 and 2448), where they were maintained on fresh cowpea plants, *Vigna unguiculata* (L.), under controlled temperature (32 ± 3°C) and a photoperiod of 16:8 (L:D) h. Insects survived in cages in this facility for 2–4 wk after field collection. Young females selected for the experiments were estimated to be 1–2 wk of age, because the first of two moderately synchronous field generations had begun to eclose to adulthood 1–2 wk previously. One indication of their young age was their egg-laying status, because they all had brochosome spots on their wings.

Four plant types commonly grown in New Zealand were selected for this experiment. They were apple, *Malus* × *domestica* Borkh. 'Fuji'; citrus (orange), *Citrus sinensis* L. 'Madam Vinous'; grape, *Vitis vinifera-*

'Cabernet Sauvignon'; and pohutukawa. Young apple trees were originally purchased from Laguna Nursery (Lakewood, CA). Grape cuttings were provided by SunRidge Nursery (Bakersfield, CA). Citrus was provided by R. Yokomi (USDA–ARS), and pohutukawa plants were purchased from Ponto's Nursery (San Diego, CA). Assurances were given by all providers that plants were pesticide-free before study (however, see Experimental Design). These plants, as well as cowpea plants, were all grown and maintained in a greenhouse at the ARS San Joaquin Valley Agricultural Science Center in Parlier, CA, under similar light and temperature regime as mentioned above. Plants were transported to the Fresno glassy-winged sharpshooter quarantine facility as needed for experiments or insect maintenance.

Pretest preparations were made 24 h before the experiment by removing two to three leaves from the dense foliage of the test pohutukawa plants near where the insect would be tethered, for ease of access. Also, young, female glassy-winged sharpshooters were transferred to acclimation cages containing healthy plants of each test species, 18–36 h before the experiments. The next day, 2–4 h before recording, test plants were set up on Plexiglas plates inside Faraday cages by using blue poster tack and Parafilm.

Experimental Design, EPG Recording, and Waveform Measurements. On the day of recording, insects were immobilized under CO_2 for 2–3 s, and then they were quickly attached to a 5–6-cm-long, 63.5-μm-diameter gold wire (sold as 0.0025 in.; Sigmund Cohn Co., Mt. Vernon, NY) with silver conducting paint (*n*-butyl acetate solvent; Ladd Research Industries, Williston, VT). After wiring, insects were left for 15–20 min to recover, and then they were placed on the test plants for EPG recording of their stylet penetration behaviors (with no other starvation period). Insects that did not probe or walked off the plant within the first hour of recordings were replaced. Insects that became detached from the wire during recording (10%) were not included in data analysis. None of the insects died during recordings.

As is typical for EPG studies (van Helden and Tjallingii 2000), the feeding site on each host plant species was standardized in the following manner, to minimize variability in data. Insects in acclimation cages were observed for 18–36 h for their preferred location on each plant type. Most insects selected and settled on the adaxial surface of leaves on pohutukawa, abaxial leaf surface of apple, and stems or petioles of citrus and grape. During EPG experiments, stubs holding wired insects were hung near these preferred areas of each plant, and relatively long tethers (3–5 cm) allowed the insects some degree of choice. Most test insects chose the same areas listed above for each plant.

Four channels of an AC-DC (alternating current-direct current) EPG monitor (E.A.B. and Bennett, unpublished data), all with a fixed input resistor level of 10^6 Ω, were used. A 25-mV, 1,000-Hz AC substrate voltage was applied to the test plant. These monitor settings are nearly identical to the settings used for previously published studies of glassy-winged sharpshooter probing (Backus et al. 2005a, Joost et al. 2006) except for lower substrate voltage. The waveform outputs were identical in appearance to published waveforms. Each morning of recording, plant types were randomly assigned to channels of the monitor and positions in the Faraday cages. The recording started with nonprobing baseline while insects were dangling, as all insects were being placed in holders and readied for recording. Then, the insects were lowered to the plants, one at a time, according to the numerical order of the channels. The temperature in the recording area was maintained at 29 ± 2°C and fluorescent lights were on during the recording period. The recording area was separated from the rest of the room by a cloth screen, so as not to disturb insects visually after they settled on the plants.

Four insects, one per day for each plant type, were monitored for nearly 20 h/d, in a randomized, complete block design. The original experimental design called for 20 replicates. However, fewer adult insects were collected than in previous years, due to cooler-than-normal spring temperatures in the timeframe given us for the project. Recordings were made for 15 d in an attempt to achieve a balanced design of 15 replicates. However, four insects that came off their wires and left plants during mid-recording (three on apple and one on citrus) were not included in the final total of 56 insects.

The waveforms were recorded on a Dell computer by using WinDaq DI-720 analog-to-digital converter and displayed with WinDaq PRO+ software (DATAQ Instruments, Akron, OH). Recordings each day began around 10:00 a.m. and proceeded for 20 h of access time until ≈6:00 a.m. the next day (actual mean recording [i.e., access] time was 1,198.5 min per insect).

Insects on all four plants showed no signs of mortality or duress. However, when most recordings were completed and waveform measurement was underway, it became clear that unusual waveforms (later termed M) were occurring more often on citrus than on other plants. This was unexpected because 'Madame Vinous' citrus is readily fed upon by glassy-winged sharpshooter (Damsteegt et al. 2006) under normal circumstances. Probing behavior on apple and pohutukawa was unknown before this work. After the recordings were nearly completed, we learned that the test citrus plants had accidentally been treated with a cocktail of insecticides, primarily imidacloprid (Admire), 6–8 wk before experiments began, although the plants had been repeatedly washed since then.

We decided to finish recordings, measure and analyze the waveforms on those contaminated citrus plants, and compare the findings with the other host plants due to unavoidable circumstances (unavailability of other citrus plants or insects during the limited time frame allowed for this project).

Waveform Designations and Interpretations. The current study followed the terminology conventions of Backus (2000). A probe is defined as all behaviors occurring from start of stylet penetration into plant

tissue until stylet withdrawal. Stylet penetration (synonymous with probing) is defined as all feeding behaviors performed during a probe. A waveform event is the duration within a probe during which an uninterrupted waveform is performed; therefore, a probe consists of an unbroken sequence of waveform events.

After data acquisition, waveform categories were assigned based on stereotypical patterns. Frequencies and amplitudes were measured following the conventions described by van Helden and Tjallingii (2000), and waveform types were identified and labeled according to Backus et al. 2005b). A summary of the biological meanings (Joost et al. 2006, Backus et al. 2005a, Holmes 2007, Dugravot et al. 2008) of the waveform types used is as follows:

Pathway Phase. A1: Movement of both mandibular and maxillary stylets with simultaneous formation of salivary sheath, especially the thick-walled trunk of the salivary sheath (Backus et al. 2005a).

B1: Penetration of the maxillary stylets past the trunk, further salivation and formation of sheath branches, fluttering of the precibarial valve to facilitate fluid tasting (E.A.B. and Dugravot, unpublished data).

B2: Maxillary stylet sawing through tough tissues or the walls of solid salivary sheath; indication of new salivary sheath branching or extension of an existing branch.

Ingestion Phase. C: Cibarial pumping for active ingestion, usually from the xylem. Correlated with cibarial dilator muscle movements and portraying streaming potentials during fluid uptake (Dugravot et al. 2008).

Interruption Phase. N: nonpathway interruption of ingestion. Stylet tips are in the xylem. Likely combined salivation, precibarial valve fluttering, and tasting of xylem contents.

NA1, NB1, and/or NB2: collectively designated pathway-type interruption: Same as A1, B1 and B2, respectively, but performed after the first C waveform event. Return to pathway activities to create a new salivary sheath branch, exiting a (presumably) unacceptable xylem cell and searching for a new cell.

Other. G: stylets motionless in the xylem with occasional precibarial valve fluttering (E.A.B., unpublished data). This is a noningestive behavior that can follow xylem ingestion, with cessation of previous excretory droplet production (Dugravot et al. 2008). This behavior seems to occur in the laboratory after several minutes to hours of xylem ingestion, perhaps because of light levels less conducive to transpiration than in greenhouse or field.

R: Flat-line waveform during which stylets are motionless in plant tissue, usually just below the epidermis. No other activity occurs, especially of the cibarial dilator muscles. R often followed G in our laboratory; it is unknown whether these resting behaviors occur in the field or only in the laboratory.

M: Highly variable mixture of waveforms resembling distorted C, G, or R. Based on previous correlations of waveforms with directionality of fluid flow due to streaming potentials (Dugravot et al. 2008), M probably represents partially paralyzed cibarial pumping, perhaps due at times to pesticide intoxication.

Z: Nonprobing/baseline. Duration measurements for each waveform event (i.e., a continuous, uninterrupted occurrence of one waveform type) were made with WinDaq/Pro+ Waveform Browser software (Serrano et al. 2000). These files were copied into a Microsoft Excel workbook developed for EPG analysis by van Giessen and Jackson (1998), and the merged dataset from all host plants was further analyzed.

Statistical Analysis. Descriptive and analytical statistics of waveforms from the experiment were performed as described by Almeida and Backus (2004) and Backus et al. (2007) by using Statistical Analysis Software (SAS Institute, Cary, NC) (SAS Institute 2001). The descriptive statistics for feeding behaviors were compiled at the heuristic levels of waveform event, probe, insect, and cohort as described in Backus et al. (2007). Nonsequential parameters for EPG data were the same as those named, described, and mathematically defined in Backus et al. (2007). Parameter acronyms can be interpreting using D for duration; E, event; I, per insect; N, number; W, waveform; P, probe or probing, also per probe; and T, total.

Repeated measures analysis of variance (ANOVA) (restricted maximum likelihood estimation; REML-ANOVA) (PROC MIXED; SAS Institute 2001) was used to determine whether the frequencies or durations of overall probing for each waveform type were significantly different, per event, per probe, per insect, or per cohort (Backus et al. 2007). Means of host plants were then separated using the LSDs test (LSMEANS option, SAS Institute 2001). Duration and frequency data were log- and square root-transformed, respectively, before ANOVA to improve homogeneity by reducing variability. Differences were considered significant at $\alpha = 0.05$.

Results from the contaminated citrus were included because they were necessary for proper statistical comparison and were part of the initial experimental design. Also, these results highlighted the type of differences in probing behavior that can be discerned via EPG. This was justified because our study was intended to be the first of several quantitative EPG comparisons among host plants for development of the Stylet Penetration Index for glassy-winged sharpshooter, not a rigorous study of the effects of insecticides on probing. Nonetheless, for comparison, we also performed ANOVA tests among the three uncontaminated host plants, excluded citrus from the comparisons. *P* values in the results tables that were still significantly different without contaminated citrus are indicated by asterisks.

Results

Waveform Analysis: Cohort Level. The overall pattern of activity during stylet probing is summarized in Fig. 1, where the total waveform duration, TWD, for each waveform type is displayed as a section of a pie that represents the access time for each host plant. Glassy-winged sharpshooters on apple and grape

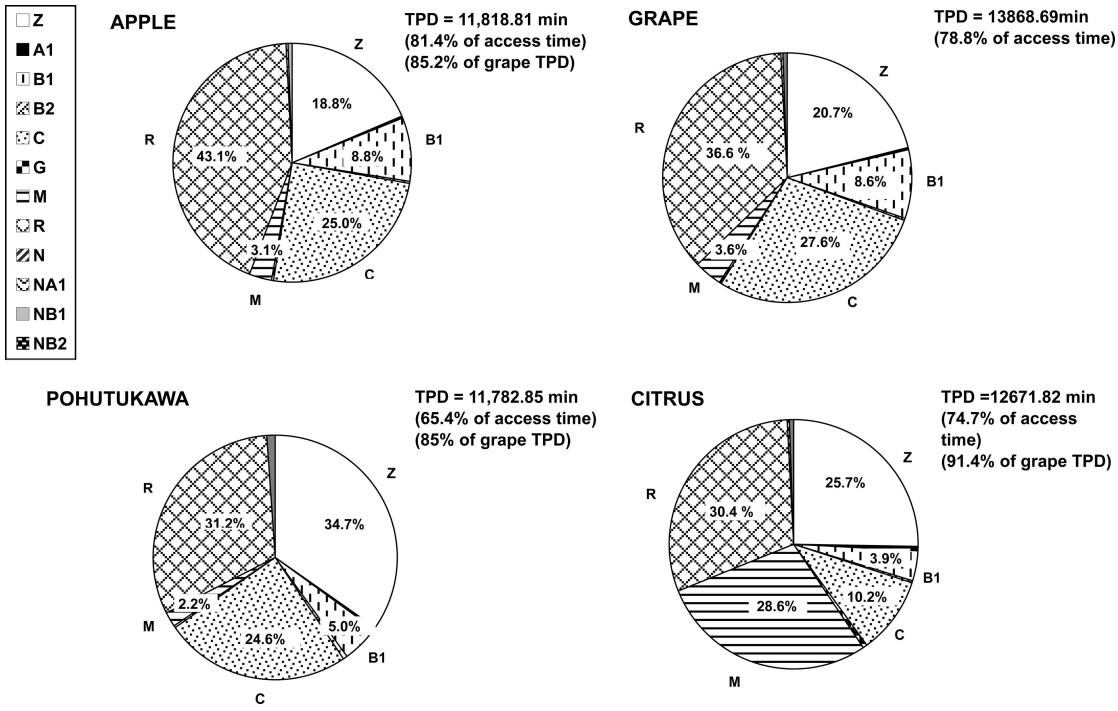

Fig. 1. TWD of probes made by glassy-winged sharpshooter for each host plant tested, displayed as sections of a pie that represents mean access time for each host. TPD, which consisted of all patterned, non-Z (white) pie sections, combined.

spent nearly the same percentage of their combined access times (81.4 and 78.8%, respectively) in stylet probing (i.e., total probing duration, TPD), whereas those on pohutukawa and contaminated citrus spent only 65.4 and 74.7%, respectively, of their time probing. The remainder of the time was spent in nonprobing activities (waveform Z in Fig. 1), chiefly standing and walking.

The major (>2% of TPD) waveforms recorded by each cohort of insects on a host plant were (in sequential order by typical performance) Z (nonprobing [white pie sections; probing waveforms are patterned]), B1 (tasting, sheath extension during pathway), C (xylem ingestion), M (partially paralyzed cibarial muscles), and R (stylets at rest). These overall patterns of waveform performance were nearly identical for apple and grape, whereas the patterns on pohutukawa and contaminated citrus were different, both from the apple and grape patterns, and from one another. Overall, insects on pohutukawa spent much less time probing than those on grape or apple, but once probing commenced, the pattern of these major waveforms was similar to that of apple and grape. In contrast, insects on contaminated citrus spent only 4.1% less time probing than on grape, but they showed a very different pattern of waveforms from that on the other three plants. Much less time was spent in B1 and C, and much more in M (Fig. 1).

In a similar manner, Fig. 2 shows pie charts for the total number of waveform events (TNWE) for each host plant. Waveform events are discussed in detail further below, under Event Level. For now, it is only necessary to observe the patterns among host plants, comparing TWD and TNWE pies. Pie sections for each waveform type are sometimes the same relative size in both TWD (Fig. 1) and TNWE (Fig. 2) pies (e.g., C in citrus), indicating that certain waveforms are both moderately long-duration and also performed very frequently. In some of the other cases, the TNWE pie sections for each waveform type are small, whereas the corresponding TWD pie sections are large (e.g., waveforms R and Z). This is because relatively infrequent events can sometimes be quite long. Alternatively, there can be many waveform events of short duration (e.g., N [salivation and precibarial valve fluttering in a xylem cell], NA1 [salivary sheath extension during pathway-type interruption, i.e., after leaving an unacceptable, usually xylem, ingestion cell], NB1 [tasting, searching, and further extension of salivary sheath branches during interruption], indicating that certain very short events can yet be very frequent. Although the total durations of these waveforms may be so very short that they are not (or barely) visible in the TPD pies, they may yet be biologically very important because of their high frequency and the nature of the preparatory behaviors they represent.

Overall, the patterns within each TNWE pies, i.e., for each host plant, relate to one another in the same manner as TWD. Apple and grape are nearly identical, the only difference being that slightly more frequent C, G, N, and NB1 events were performed on apple than on grape, whereas slightly more A1, B1, and B2 events occurred on grape compared with apple. Pohutukawa had more frequent B1 and B2 than apple

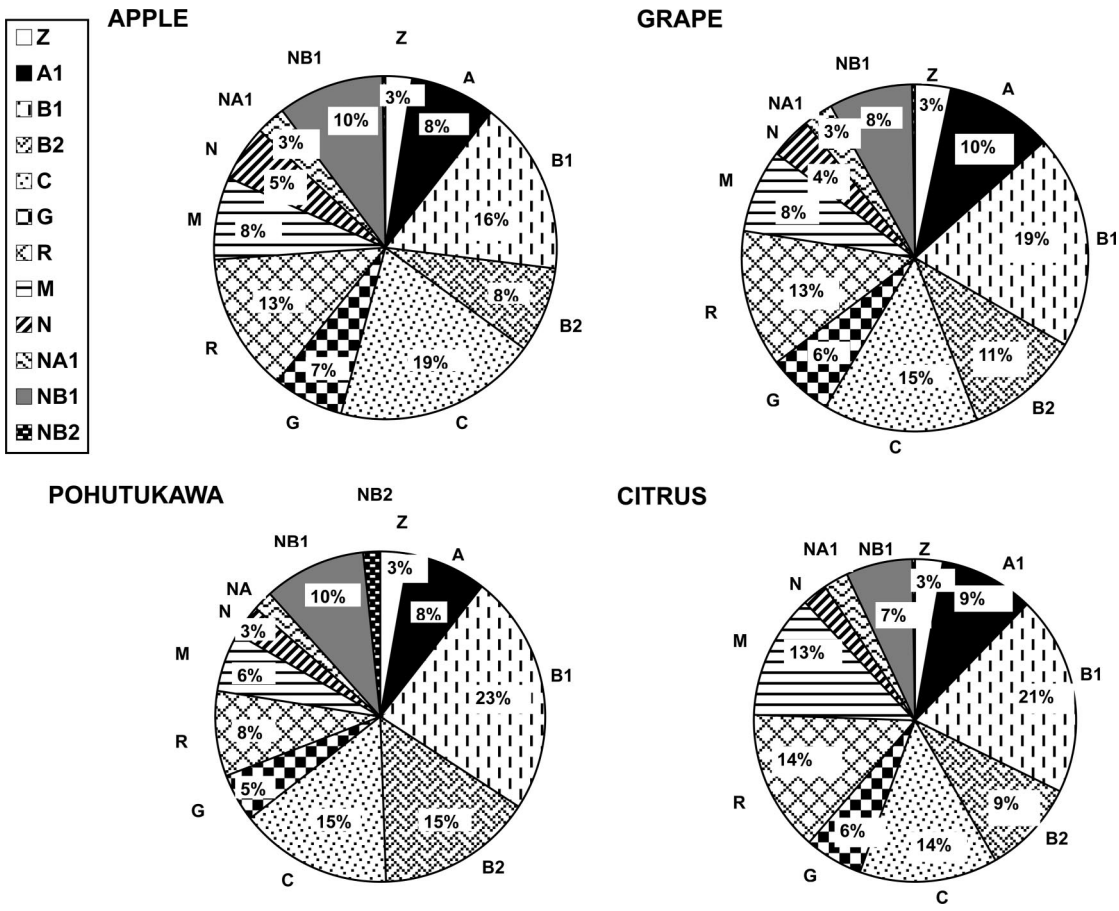

Fig. 2. TNWE of probes made by glassy-winged sharpshooter for each host plant tested, displayed as sections of a pie that represents TNPE for each host.

and grape, whereas contaminated citrus had fewer C and more M than apple and grape.

Thus, even such a gross overview of EPG data reveals patterns of differences and similarities among host plants, suggesting that apple and grape are similar, whereas pohutukawa and contaminated citrus are very different, both from one another and from apple and grape. To test this hypothesis more rigorously, we subdivided this cohort-level analysis into insect, probe, and event levels, to test the mean durations and frequencies of these waveform measurements statistically. First, we examined the smallest possible unit, the waveform events, and then built back to the cohort level via probe and insect levels.

Waveform Analysis: Event Level. A waveform event is the shortest, continuous duration of a certain waveform type. When all events are combined together, regardless of waveform type, they are termed probing events (or nonprobing events, in the unique case of waveform Z). Mean probing durations per event (PDE) were highly significantly different among host plants ($F = 39.51$, df = 3, $P < 0.0001$). The longest probing events were made on apple, followed by contaminated citrus (significantly different between them). Grape and pohutukawa, which were not significantly different from each other, yielded the shortest events (Table 1).

There were many differences among the host plants in mean waveform durations per event (WDE) (Table 2); only waveforms B1 and NB2 (stylet sawing to cut a new sheath branch during pathway-type interruption) were not significantly different among host plants. In addition, the significant differences among hosts form a distinct pattern. The significantly longest events of each waveform type (including Z) almost always occurred on either pohutukawa or contaminated citrus, whereas the shortest events occurred on apple or grape. The only exception to this observation was for waveform C (xylem ingestion), for which the longest event duration occurred on grape, intermediate durations on apple and pohutukawa, and the shortest on contaminated citrus.

When all pathway waveforms were combined (i.e., waveforms A1, B1, and B2), mean pathway events were significantly different ($F = 3.54$, df = 3, $P = 0.0141$), with the longest combined pathway activities on apple, the shortest on pohutukawa. Likewise, when all pathway-type interruption waveforms were com-

Table 1. Mean or total probing durations (minutes) and frequencies ± SEM of nonsequential parameters for EPG data recorded on each plant type

Variable name (abbreviation)	Apple (%)	Grape (%)	Pohutukawa (%)	Citrus (%)	*P* value
Total access time (TAT)	14,523.6	17,591.3	18,008.5	16,970.4	
Total no. of nonprobing events	86 (2.5)	139 (3.1%)	122 (2.7)	107 (2.8)	
Total probing duration (TPD)	11,818.8 (81.4)	13,868.7 (78.8)	11,782.9 (65.4)	12,671.8 (74.7)	
Total no. of probing events (TNPE)	3400 (97.5)	4335 (96.9)	2242 (94.8)	3739 (97.2)	
Probing duration per event (PDE)	3.48 ± 0.23a	3.20 ± 0.20b	2.66 ± 0.26b	3.39 ± 0.23c	<0.0001*
Probing duration per insect (PDI)	984.9 ± 74.0	924.6 ± 79.0	785.5 ± 58.5	905.1 ± 79.5	0.4406
No. of probing events per insect (NPEI)	283.3 ± 42.3	289.0 ± 45.2	294.9 ± 26.3	267.1 ± 36.9	0.3768
No. of probes per insect (NPI)	7.25 ± 1.14	9.13 ± 1.39	8.07 ± 1.08	7.57 ± 1.15	0.4739
Probing duration per probe (PDP)	135.8 ± 18.9a	101.2 ± 16.8b	97.4 ± 17.9b	119.5 ± 18.8c	<0.0001*

Values in the same row with the same letters indicate that the means are not statistically different.
* *P* values that remain significant (<0.05) when citrus is removed and only the three uncontaminated host plants are compared via ANOVA.

bined (waveforms NA1, NB1 and NB2), mean duration was again significant ($F = 20.02$, df = 3, $P < 0.0001$). However, the longest interruption activities were performed on pohutukawa and contaminated citrus, whereas the shortest were on grape (Table 2).

Yet, when frequencies of these waveform events were counted and averaged per probe, none of the waveform types showed significant differences among hosts (Table 3, mean number of waveform events per probe, NWEP). Thus, within an average probe, the number of each type of waveform event performed was somewhat stereotypical; only the durations of those events changed significantly from host to host. When mean numbers of waveform events per insect (NWEI) were calculated (Table 4), only two waveform types differed significantly among host plants: NB1 ($F = 2.78$, df = 3, $P = 0.0502$) and NB2 ($F = 4.79$, df = 3, $P = 0.0156$). NB1 events were much less frequent on contaminated citrus, most frequent on pohutukawa, and intermediate on apple and grape. NB2 was very highly frequent on pohutukawa, and much less common (not significantly different) on the other three host plants (Table 4). This suggests that NWEP for those two waveforms, which numerically seem to vary greatly, might have been significantly different if a larger sample size of insects were recorded. Thus, some slight variation in number of waveform events also might account for differences in overall probing differences (TWD) among host plants, in addition to the contribution from differing WDE. This also confirms the importance of these interruption behaviors as a major difference between pohutukawa and the other host plants. Because these waveform events comprise the insects' probes, the above-mentioned data on events can be further applied to analysis of the probes.

Waveform Analysis: Probe Level. Similar to the event level data, the mean probing duration per probe (PDP) was highly significantly different ($F = 39.5$, df = 3, $P < 0.0001$) among host plants (Table 1). The average probe on pohutukawa was much shorter than on all other hosts; it was significantly shorter than both the very long probes on apple and long probes on contaminated citrus (which were significantly different from each other), but it was not significantly different from mean probe duration on grape (Table 1). This again supports that probing behavior is quite different on pohutukawa, compared especially with apple and citrus. Also, the rank order of PDP, from shortest durations (on grape and pohutukawa) to longest (on apple) was the same as the order of PDE (Table 1). This supports that the differences among hosts seen at the probe level are caused by differences at the event level.

Table 2. Mean durations (minutes) per event ± SEM for main waveform types on each plant type

Waveform		Waveform duration (WDE)				*P* value
		Apple	Grape	Pohutukawa	Citrus	
Z	Nonprobing	31.5 ± 11.33a	26.8 ± 7.62a	51.2 ± 9.62b	40.2 ± 11.3b	<0.0001
A1	Pathway	0.12 ± 0.01a	0.13 ± 0.01b	0.16 ± 0.01c	0.16 ± 0.01abc	0.0375
B1	Pathway	2.28 ± 0.62	1.81 ± 0.42	0.86 ± 0.13	0.88 ± 0.13	0.9054
B2	Pathway	0.13 ± 0.01a	0.12 ± 0.00b	0.14 ± 0.00c	0.18 ± 0.01d	<0.0001
C	Ingestion	5.56 ± 0.44a	7.91 ± 0.74b	6.68 ± 1.33a	3.24 ± 0.29c	0.0004
G	Resting	0.22 ± 0.02a	0.17 ± 0.02b	0.24 ± 0.02ac	0.41 ± 0.07c	<0.0001
R	Resting	14.23 ± 1.30a	11.92 ± 1.01b	15.24 ± 1.83b	9.80 ± 0.86b	0.0568
M	Intoxication	1.65 ± 0.34a	1.92 ± 0.37a	1.51 ± 0.35a	10.12 ± 1.39b	<0.0001
N	Nonpathway interruption	0.12 ± 0.01a	0.15 ± 0.01b	0.16 ± 0.01c	0.24 ± 0.04d	<0.0001
NA1	Pathway-type interruption	0.12 ± 0.01a	0.13 ± 0.01ab	0.19 ± 0.03c	0.16 ± 0.02bc	0.0040
NB1	Pathway-type interruption	0.29 ± 0.24b	0.25 ± 0.02a	0.38 ± 0.02c	0.36 ± 0.02c	<0.0001*
NB2	Pathway-type interruption	0.12 ± 0.02	0.08 ± 0.01	0.13 ± 0.01	0.14 ± 0.04	0.0608
Combined A1, B1, B2		1.20 ± 0.32ab	0.95 ± 0.21b	0.48 ± 0.06c	0.52 ± 0.07b	0.0141
Combined NA1, NB1, NB2		0.25 ± 0.02a	0.21 ± 0.01c	0.32 ± 0.02b	0.29 ± 0.02b	<0.0001

Values in the same row with the same letters indicate that the means are not statistically different.
* *P* value that remains significant (<0.05) when citrus is removed and only the three uncontaminated host plants are compared via ANOVA.

Table 3. Mean number of events per probe ± SEM for each main waveform type on each plant type

Waveform		No. waveform events per probe (NWEP)				*P* value
		Apple	Grape	Pohutukawa	Citrus	
A1	Pathway	3.23 ± 0.28	3.21 ± 0.23	2.85 ± 0.17	3.43 ± 0.24	0.8412
B1	Pathway	7.09 ± 1.14	6.33 ± 0.85	8.83 ± 1.04	7.66 ± 0.82	0.4205
B2	Pathway	7.69 ± 2.02	8.62 ± 1.67	8.04 ± 1.26	7.67 ± 1.12	0.2269
C	Ingestion	16.6 ± 2.33	11.0 ± 01.21	10.2 ± 1.08	10.5 ± 1.27	0.7712
G	Resting	7.89 ± 1.08	8.19 ± 1.62	7.25 ± 1.84	7.93 ± 2.16	0.5485
R	Resting	11.3 ± 1.35	14.2 ± 2.65	10.3 ± 1.89	10.1 ± 2.00	0.9225
M	Intoxic	8.47 ± 1.05	8.54 ± 1.63	8.90 ± 1.52	7.51 ± 1.29	0.4517
N	Nonpathway interruption	6.36 ± 1.64	4.57 ± 0.94	2.91 ± 0.37	3.17 ± 0.60	0.4899
NA1	Pathway-type interruption	3.54 ± 0.59	3.85 ± 0.69	2.59 ± 0.35	2.90 ± 0.37	0.8368
NB1	Pathway-type interruption	9.57 ± 1.31	6.80 ± 0.91	7.81 ± 0.80	5.93 ± 0.59	0.4425
NB2	Pathway-type interruption	2.50 ± 0.87	1.75 ± 0.48	3.24 ± 0.59	1.86 ± 0.46	0.3205

Interestingly, the mean number of probes per insect (NPI) was not significantly different among hosts (Table 1), although numerically the numbers of probes on both apple and contaminated citrus were slightly lower. The numerical difference between frequency of probes on apple v. grape suggests there might be a trend toward slightly fewer probes of significantly longer duration on apple than on grape; otherwise, the two hosts continue to seem quite similar. Accordingly, the differences at the cohort level are more likely to arise from durations of probes, even though the number of probes may vary so slightly that a trend might become significant with much larger sample size. This is further suggested by analysis of types of probes.

Glassy-winged sharpshooter probes can be segregated into those with short durations (<5.0 min), medium (≥5.0–30 min), or long durations (≥30 min) (Fig. 3; Table 5). Short probes consist exclusively of pathway activities (Fig. 3, top inset), and they represent test probes that probably function in host plant acceptance (Backus 1988). Medium probes consist of pathway plus alternating short events of ingestion and interruption phases, together termed trial ingestion (Fig. 3, top inset). Medium probes are usually terminated after only a short duration of trial ingestion; thus, functioning as test probes for acceptability of xylem. Long-duration probes usually have longer pathway phases than do medium probes. They include more trial ingestion followed by longer ingestion events that characterize sustained ingestion, which then can continue for several hours (Fig. 3, bottom inset). Although the mean durations of long probes were between 3.75 and 4 h, they were highly variable in their ultimate length. The longest probes on each host plant varied from 10 to 18 h (Table 5). In total, 1,447 short probes were performed, 387 medium probes, and 99 long probes (Fig. 4).

When NPI and PDP values were recalculated for each probe type (Table 5), their statistical comparison further supported differences among host plants. The mean durations (PDP) of short probes were significantly different among hosts ($F = 4.95$, df = 3, $P = 0.0027$). Short probes of apple and grape probes were not significantly different from one another. However, both were significantly shorter than pohutukawa and contaminated citrus probes, which were not significantly different from each other. Medium and long probes did not differ in duration among host plants. In contrast, the same number of probes (NPI) was made on all host plants within short and long probe categories. However, mean durations of medium-length probes were significantly different among hosts ($F = 3.24$, df = 3, $P = 0.0257$), chiefly because of more medium probes on pohutukawa (Table 5).

Thus, differences at the probe level among the host plants were due to 1) significantly longer (yet no more

Table 4. Mean number of events per insect ± SEM for main waveform types on each plant type

Waveform		No. waveform events (NWEI)				*P* value
		Apple ($n = 12$)	Grape ($n = 15$)	Pohutukawa ($n = 15$)	Citrus ($n = 14$)	
Z	Nonprobing	7.17 ± 1.18	9.27 ± 1.35	8.13 ± 1.06	7.64 ± 1.08	0.797
A1	Pathway	22.3 ± 4.63	29.1 ± 5.26	22.88 ± 2.56	24.71 ± 4.68	0.4895
B1	Pathway	46.1 ± 9.18	57.0 ± 13.1	69.5 ± 11.0	53.6 ± 9.72	0.2753
B2	Pathway	25.2 ± 6.67 (11)[a]	30.5 ± 9.21	44.9 ± 8.77	25.2 ± 5.30	0.2666
C	Ingestion	53.8 ± 8.78	41.7 ± 5.19	44.2 ± 3.51	37.6 ± 5.21	0.2693
G	Resting	20.1 ± 5.86 (11)	20.2 ± 5.62 (13)	14.5 ± 3.25 (14)	18.5 ± 5.78 (12)	0.9117
R	Resting	36.7 ± 8.95	38.6 ± 9.66 (14)	24.7 ± 4.27	40.5 ± 9.34 (13)	0.7097
M	Intoxic	24.6 ± 5.50 (11)	25.6 ± 6.41 (13)	17.2 ± 3.38	33.8 ± 6.84 (14)	0.2389
N	Nonpathway interruption	14.8 ± 4.84	13.0 ± 5.08 (13)	8.93 ± 1.46 (12)	7.67 ± 1.84	0.5672
NA1	Pathway-type interruption	8.36 ± 1.69 (11)	8.47 ± 1.63	5.53 ± 1.25	6.92 ± 1.93 (13)	0.3796
NB1	Pathway-type interruption	27.9 ± 4.44b	22.2 ± 2.31bc	29.7 ± 3.30c	17.8 ± 2.4a	0.0502
NB2	Pathway-type interruption	2.50 ± 0.87a (4)	2.33 ± 0.88a (3)	11.3 ± 3.17b (6)	2.17 ± 0.98a (6)	0.0156*

Values in the same row with the same letters indicate that the means are not statistically different.

[a] Numbers in parentheses are the number of insects when that number is different from the cohort number per host plant [*n*].

* *P* value that remains significant (<0.05) when citrus is removed and only the three uncontaminated host plants are compared via ANOVA.

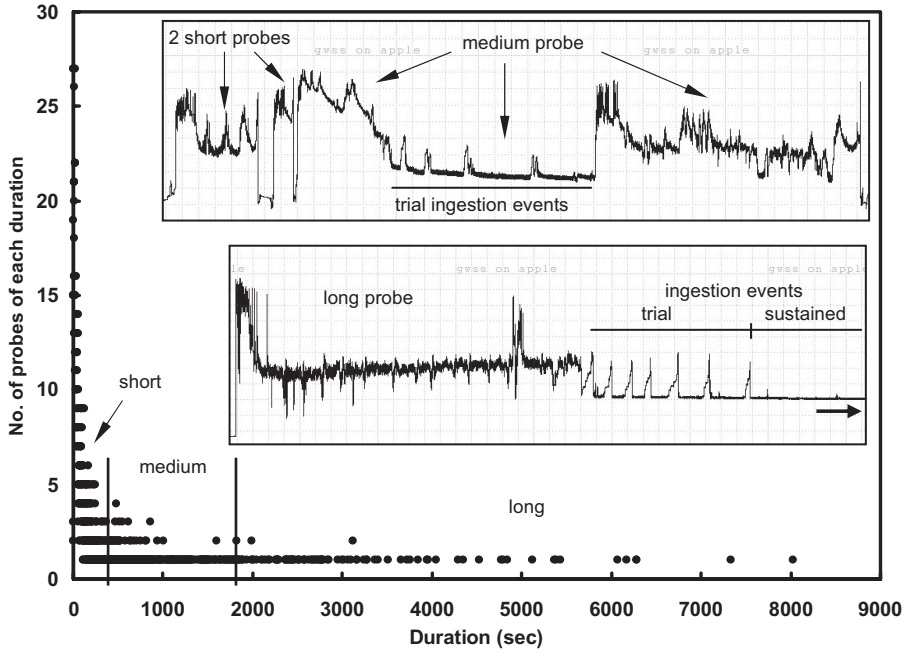

Fig. 3. Frequency distribution of all probes made by glassy-winged sharpshooter performed on the four host plants in this study. Vertical lines indicate the divisions between short, medium and long probes. Inset boxes show typical EPG waveforms for each of the three probe types. Top box, two short probes (149 and 35 s in duration, respectively) followed by a medium-duration probe of 1,027 s (17.1 min) duration. Bottom box, one long probe showing 1,212 s (20.2 min) of a total 54,274 s (15.1 h). All waveforms shown at Windaq compression of 150 (30 s per division).

frequent) short (host-test) probes being made on pohutukawa and contaminated citrus than on apple and grape, and 2) a larger number (yet no longer in duration, on average) of medium (xylem-test) probes made on pohutukawa than on other plants.

The behaviors that occurred during all, combined, types of probes (or between them, as with waveform Z), summed across all events in the probe, are summarized in Fig. 4a (for waveforms with short durations, i.e., <12 min) and Fig. 4b (for waveforms with long durations, i.e., from 50 to 600 min). These mean waveform durations per probe (WDP) were only significantly different for the following three waveform types: 1) G, stylets resting in the xylem ($F = 2.85$, df = 3, $P = 0.0479$), whose duration on contaminated citrus was nearly 5 times as long as grape, with apple and pohutukawa intermediate (Fig. 4a), 2) NB1 ($F = 3.34$, df = 3, $P = 0.0259$), whose duration on pohutukawa was significantly more than twice as long as the shortest on grape, with both apple and contaminated citrus intermediate (Fig. 4a), and 3) M ($F = 7.41$, df = 3, $P = 0.0003$), whose length on contaminated citrus was greatly (10 times longer) elongated compared with all other hosts, which were not significantly different

Table 5. Number of probes per insect (NPI) and the probing duration per probe (PDP) segregated into short, medium, and long probes, with given durations

Probe length		Apple	Grape	Pohutukawa	Citrus	*P* value
Short (1–5-min) range	NPI	3.00 ± 0.65 1–7	4.27 ± 0.74 1–8	3.36 ± 0.80 1–8	2.78 ± 0.86 1–8	0.2692
	PDP	0.89 ± 0.17a (27)[a] 0.12–3.30	1.32 ± 0.20a (47) 0.13–4.87	1.88 ± 0.21b (37) 0.10–4.65	1.82 ± 0.28b (25) 0.08–4.65	0.0027*
Medium (5–30-min) range	NPI	2.20 ± 0.49a 1–4	2.50 ± 0.42a 1–5	2.85 ± 0.59b 1–8	2.08 ± 0.43a 1–5	0.0029*
	PDP	12.7 ± 2.28 (11) 5.85–27.8	13.8 ± 1.20 (35) 5.01–29.2	13.4 ± 1.08 (37) 5.01–29.8	13.9 ± 0.94 (27) 5.23–23.8	0.7728
Long (>30-min) range	NPI	4.08 ± 0.42 2–7	3.67 ± 0.56 1–8	3.13 ± 0.27 1–5	3.86 ± 0.43 2–7	0.7945
	PDP	237.9 ± 25.4 (49) 30.45–639.0	242.3 ± 34.0 (55) 32.0–1103.4	238.6 ± 38.0 (47) 30.6–987.9	226.9 ± 30.4 (54) 31.2–919.6	0.6163

Values in the same row with the same letters indicate that the means are not statistically different.

[a] Numbers in parentheses are the number of probes of that length made on that host plant.

* *P* values that remain significant (<0.05) when citrus is removed and only the three uncontaminated host plants are compared via ANOVA.

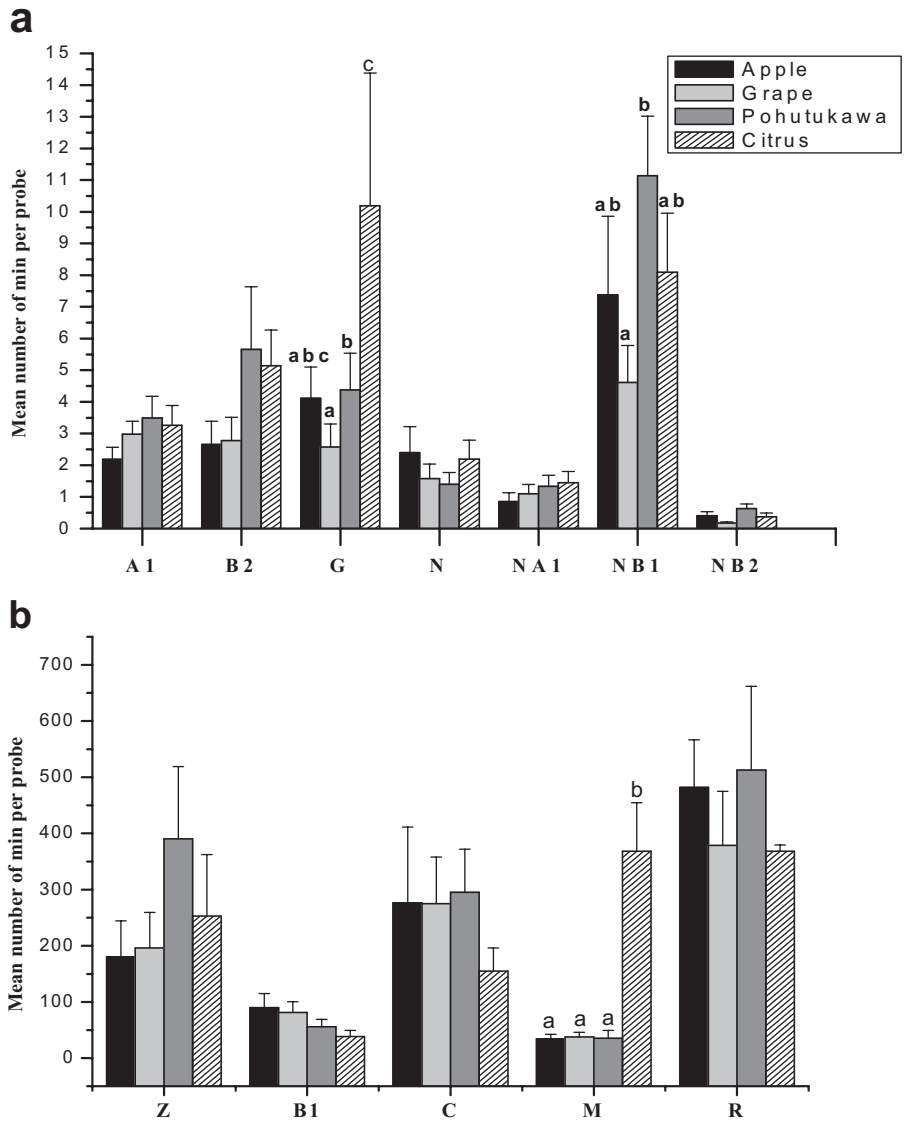

Fig. 4. The mean WDP made by glassy-winged sharpshooter on the four host plants tested. (a) For waveforms with short mean durations (1–16 s) per probe. (b) For waveforms with long mean durations (1–700 s) per probe.

(Fig. 4b). Thus, just as numbers and durations of events combine to be reflected in numbers and durations of probes, per probe data combine to give insights per insect.

Waveform Analysis: Insect Level. The durations of all stylet penetration behaviors, combined for all insects then averaged per insect, did not differ significantly among host plants, as shown by the mean probing duration per insect (PDI) (Table 1). Numerically, there may be a trend that insects on pohutukawa probed for a shorter duration (Table 1), that might have become significant had we recorded a higher sample size of insects. However, PDI was very similar on the other host plants. Likewise, the mean number of probing events per insect (NPEI) was not significantly different among host plants although, numerically, the contaminated citrus had fewer probing events performed on it, whereas the other hosts were nearly identical. Thus, when combined probing events were compared at the insect level, few differences were seen among hosts. This shows that the frequency and duration differences seen at the event and probe levels compensated for one another at the insect level, when all waveform types were combined. However, this was no longer the case when the per-insect data were separated by waveform type.

Mean waveform duration per insect (WDI) showed that although lengths of most waveform events were not significantly different among hosts, the following four biologically important waveforms did vary sig-

Table 6. Mean durations (minutes) per insect ± SEM for the main waveform types on each plant type

Waveform		Waveform duration per insect (WDI)				*P* value
		Apple (n = 12)	Grape (n = 15)	Pohutukawa (n = 15)	Citrus (n = 14)	
Z	Nonprobing	225.4 ± 72.7	248.2 ± 78.9	416.5 ± 59.2	307.3 ± 77.2	0.0882
A1	Pathway	2.74 ± 0.53	3.77 ± 0.68	3.72 ± 0.49	3.96 ± 0.80	0.4057
B1	Pathway	105.0 ± 40.9	103.2 ± 30.3	60.0 ± 12.0	47.0 ± 11.2	0.8819
B2	Pathway	3.14 ± 0.93 (11)[a]	3.52 ± 0.93	6.03 ± 1.27	4.41 ± 1.16	0.2862
C	Ingestion	299.4 ± 71.2a	330.1 ± 70.5a	295.3 ± 73.2a	121.8 ± 25.4b	0.0269
G	Resting	4.48 ± 1.37 (11)	3.37 ± 0.81 (13)	3.44 ± 0.74 (14)	7.64 ± 3.61 (12)	0.7430
R	Resting	521.9 ± 80.9	459.7 ± 75.1 (14)	375.9 ± 53.1	396.6 ± 84.7 (13)	0.8939
M	Intoxic	40.6 ± 16.7a (11)	49.1 ± 13.7a (13)	25.9 ± 8.11a	342.0 ± 70.9b	<0.0001
N	Non-pathway interruption	1.80 ± 0.50	1.95 ± 0.76 (13)	1.40 ± 0.30 (12)	1.83 ± 0.38	0.7816
NA1	Pathway-type interruption	1.01 ± 0.15 (11)	1.10 ± 0.21	1.07 ± 0.29	1.12 ± 0.34 (13)	0.7304
NB1	Pathway-type interruption	7.99 ± 1.44abc	5.53 ± 0.96a	11.14 ± 1.29c	6.34 ± 0.86b	0.0097*
NB2	Pathway-type interruption	0.30 ± 0.13ab (4)	0.18 ± 0.09a (3)	1.48 ± 0.37b (6)	0.31 ± 0.12a (6)	0.0469*
Combined A1, B1, B2	Pathway-type interruption	138.1 ± 76.1	140.1 ± 81.9	53.8 ± 23.3	62.0 ± 30.5	0.8388
Combined NA1, NB1, NB2	Pathway-type interruption	9.40 ± 2.68ab	8.44 ± 2.65a	11.98 ± 2.54b	8.38 ± 2.54a	0.0121*

Values in the same row with the same letters indicate that the means are not statistically different.

[a] Numbers in parentheses are the number of insects when that number is different from the cohort number per host plant [n].

* P values that remain significant (<0.05) when citrus is removed and only the three uncontaminated host plants are compared via ANOVA.

nificantly among hosts (Table 6): 1) C (F = 3.32, df = 3, P = 0.0269), which was much shorter on citrus than on other hosts, among which there was no difference; 2) M (F = 10.28, df = 3, P < 0.0001), whose duration was greatly lengthened on contaminated citrus, but not different among the other hosts; 3) NB1 (P = 0.0097; F = 4.21; df = 3), which was much longer on pohutukawa, short on grape, and intermediate on contaminated citrus and apple (Table 6); 4) NB2, (P = 0.0459; F = 3.36; df = 3), was shortest on pohutukawa, intermediate on grape and apple and longest on contaminated citrus.

Summary of Waveform Analysis. Of the 74 different parameters of glassy-winged sharpshooter stylet penetration tested via ANOVA, 26 were significantly different among host plants; 12 of these were for WDE. Thus, the variation in probing behaviors among host plants was caused primarily by strongly significant differences in durations at the waveform event level. Interestingly, almost no significant differences occurred among hosts in the frequency (number) of waveform events per probe. Only numbers of pathway-type interruption events (NB1 and NB2) were significantly different per insect. Nonetheless, numerical differences in frequencies at the probe level may represent differences among hosts that would have been significant with higher sample sizes. If so, then frequencies of waveform events would have compensated for differences in durations per event, explaining the few significant differences in waveform durations when they were compiled per probe and per insect. A few, but important, significant differences occurred in waveform durations per insect, especially in cibarial activities (ingestion [C] v. partial cibarial paralysis [M]) and in searching for new xylem cells after rejection of the first tasted xylem cell (pathway interruption behaviors [NB1 and NB2]).

When contaminated citrus was removed from the dataset and statistical analyses performed again, only nine of the 26 significantly different ANOVA results were retained (see asterisks near p vales in the tables). Of the 17 lost significances, 11 were in waveform durations per event (WDE, Table 2); WDE had had the majority of differences with citrus data present. Nonetheless, the nine significant differences left were important, e.g., all of the significant probing durations and frequencies (for all probe types and for short probes), as well as durations of the interruption waveforms NB1 (per event and per insect), NB2 (per insect), and combined NB1 plus NB2 (per insect). Also, frequency of events of NB2 and short probes per insect remained significant. Thus, the fundamental conclusion remains that the differences among the uncontaminated host plants occurred primarily in durations of interruption, i.e., xylem searching behaviors after rejection of the first xylem cell. Loss of sample size by removing the citrus data removed the ability to pinpoint the mechanism of difference among the host plants as changes in durations of individual events, seen with the increased sample size when citrus data are added back. This finding further highlights the fine-scale analytical capabilities of EPG studies.

Discussion

We have shown that quantitative analysis of electrical penetration graph (EPG) waveforms is a powerful means of assessing the probing behaviors of the glassy-winged sharpshooter on four selected plant types. This study has applied statistical methods whose results will become the first of several data sets used to develop the glassy-winged sharpshooter Stylet Penetration Index (SPI). Although xylem ingestion ultimately occurred on all plant types tested, patterns of probing behaviors revealed distinct differences in amount of searching for and acceptance of xylem among these plant types. Grape has been recorded as one of the main host plants of glassy-winged sharpshooter (Hoddle 2003). However, this is the first quantitative EPG study of glassy-winged sharpshooter

comparing feeding behavior on grape versus three other plant species.

Summary of Probing Behaviors on Each Plant Type. *Apple and Grape.* Insects on apple spent 81.4% of their access time in probing, with the longest probing duration per probe. The probing duration on grape was 77.2% of the access time, and the duration per probe was significantly shorter than on apple. The durations of nonprobing (Z) and pathway-type interruption (combined NA1, NB1, and NB2) on apple and grape were shorter than on pohutukawa and citrus. Thus, although ease of xylem finding and acceptance was slightly greater on apple, overall both apple and grape presented fewer obstacles to ingestion compared with either pohutukawa or contaminated citrus, as judged by the long durations of xylem ingestion per insect.

Pohutukawa. Although xylem ingestion on pohutukawa was similar to apple in both duration and frequency, pohutukawa produced the shortest pathway phase, consisting of longer events of A1 (sheath trunk formation at the beginning of the probe), but short durations of both B1 (salivation and tasting) (numerically) and B2 (stylet sawing and sheath branching) (significantly). In contrast, NA1 and NB1 were very long (significantly), leading to the longest interruption phase.

Nonpathway interruption (N) is an important waveform because of recent findings revealing that each N event is part of the sharpshooter X wave (Holmes 2007, Backus 2007), the EPG term for a stereotypical waveform that definitively identifies first xylem penetration. The N portion of the X wave contains subphases that represent a combination of egestion and salivation behaviors apparently involved in tasting (Joost et al. 2006). Thus, N is hypothesized to be involved in *X. fastidiosa* inoculation (Backus 2007). Probing on pohutukawa resulted in N events that were significantly longer in duration than those on apple or grape, although shorter than on contaminated citrus. In addition, the number of N waveform events per probe on pohutukawa was numerically the lowest of all host plants. Thus, although each N event was longer in duration, they were infrequent enough that the N duration per insect was shorter (numerically) than on apple or grape. Future research will determine whether such differences in N duration and frequency effect *X. fastidiosa* inoculation success. If so, such information, plus host plants such as pohutukawa, will be important for development of the SPI (see below).

The probing duration per short probes (1–5 min) on pohutukawa was longer than on both apple and grape. These test probes function to decide acceptance of the host plant (Backus 1988) and to locate and test a xylem ingestion cell (Holmes 2007). Thus, glassy-winged sharpshooter required a longer time to accept pohutukawa for further probing. Significantly more medium-duration probes (5–30 min) and numerically (though not significantly) fewer long probes (>30 min) were made on pohutukawa. Medium-length probes included testing of the xylem, but were usually terminated before sustained ingestion in long probes. Thus, insects on pohutukawa took little time to reach the first xylem cell, but often rejected it, and thereafter spent a significantly longer time during a single probe searching for an acceptable xylem cell. Repeated probes with longer searching and testing after the first xylem contact were needed before a xylem cell was finally accepted. Once an acceptable xylem cell was found, however, the ingestion that ensued lasted for the same durations (per probe and per insect) as on apple. Thus, pohutukawa was a much less acceptable host plant for glassy-winged sharpshooter. Free-ranging insects (not tethered, as in EPG) might choose to depart from such a host, an option not given an insect during EPG.

Residual Insecticide-Contaminated Citrus. Total probing duration on contaminated citrus was not very different from other hosts. However, partially paralyzed cibarial movements (waveform M), occurred for a significantly longer period on citrus than on any other plant, presumably because of accidental, sublethal pesticide intoxication. In addition, contaminated citrus produced longer events of B2, G, N, and NB1. Pathway events were intermediate in length, with some searching and extra branching to get to the first xylem cell (B2). Like pohutukawa, long interruptions on contaminated citrus demonstrate more searching for and reluctance to accept xylem cells (N, and NB1). Unlike pohutukawa, however, very long, abnormally noningestive, resting of stylets (G and M), accompanied severe reduction of xylem ingestion. The contaminated citrus thus led to lack of acceptance of xylem for glassy-winged sharpshooter ingestion.

Anecdotal observations in the field, as well as published studied (Damsteegt et al. 2006), strongly support that 'Madame Vinous' orange citrus is a highly accepted host plant for glassy-winged sharpshooter feeding. Indeed, we included citrus in our comparison because we assumed that it would provide a susceptible check. We were surprised to see the waveform differences described above. Nonetheless, implications of our findings are interesting and may be very relevant to field observations today. Our findings suggest that glassy-winged sharpshooter feeding can be highly affected by sublethal, residual traces of imidacloprid and other insecticides being used in for areawide management in California now. Such areawide management has been very efficacious in decreasing glassy-winged sharpshooter populations in the field using only a single, springtime application of insecticide. This may in part be due to the significant reduction in xylem acceptance by glassy-winged sharpshooter seen and therefore possibly inoculation of *X. fastidiosa.* Our work supports that a future EPG study, using more rigorously controlled applications of sublethal insecticide concentrations, world be valuable in explaining field observations.

Background on Development of a Glassy-Winged Sharpshooter SPI. The primary application of the current study is to test methods and produce the first of several data sets that will be meta-analyzed to develop an SPI for glassy-winged sharpshooter. An SPI is a type of resistance index based solely on EPG data that can

be used for screening crop genotypes for host plant resistance, in the case of glassy-winged sharpshooter, to the *X. fastidiosa* inoculation behavior. The concept of an SPI was introduced by Backus and colleagues, who successfully developed such an index for *Empoasca* spp. leafhoppers whose direct feeding damage causes yield-reducing injury called hopperburn (reviewed in Backus et al. 2005b).

Backus and coworkers developed the *Empoasca* SPI through three main steps, which are being repeated for her work on glassy-winged sharpshooter. In the *Empoasca* work, first they performed basic research to correlate and define *Empaosca* waveforms (Hunter and Backus 1989). They found that *Empoasca* performs three different tactics of stylet probing behaviors whose proportions within total probing vary from host plant to host plant (Serrano et al. 2000). Analogous studies for glassy-winged sharpshooter, i.e., characterization and definition of EPG waveforms, now have been completed and are either published or are being written for publication (Backus et al. 2005a, Joost et al. 2006, Holmes 2007, Dugravot et al. 2008).

In the second step developing the *Empoasca* SPI, researchers determined how the most damaging of the stylet penetration tactics causes injury to host plants (Ecale Zhou and Backus 1999) and that different tactics cause different symptoms of hopperburn (Serrano and Backus 1998). This was important because symptoms and their severity vary from host species to species. Similar work for glassy-winged sharpshooter and *X. fastidiosa* is nearly completed, i.e., to determine the mechanism of transmission and correlate that with specific EPG waveforms, representing especially the inoculation behavior.

For the third step in developing the *Empoasca* SPI, researchers compared probing behaviors on a variety of more-accepted versus less-accepted, and resistant versus susceptible host plants. They found that performance of the most damaging probing tactics differed on resistant versus susceptible plants (Serrano et al. 2000). They then developed and used multivariate statistical analysis methods to distill EPG measurements of probing into a single value between 0.0 and 2.0, the SPI (Serrano et al. 2000, Backus et al. 2005b). Previous work by bean breeders had developed a field yield-based resistance index that was very time-consuming and labor-intensive to generate, and involved destructive sampling of plants. Comparison of the yield-based resistance index with the SPI showed that the SPI could replicate the yield index with a technique, EPG, that required only a few hours of nondestructive sampling of plants (Serrano et al. 2000, Backus et al. 2005b).

The present research is the first quantitative statistical step toward a similar SPI for resistance to *X. fastidiosa* inoculation behavior by glassy-winged sharpshooter. Once the waveform representing inoculation is identified, the present results plus others underway will be meta-analyzed to determine the exact statistical parameters that will be included in the multivariate analyses. The wider the tested array of host plants from agricultural systems under threat by glassy-winged sharpshooter worldwide, the more widely applicable will be the SPI, a unique contribution of EPG research to agriculture.

Implications of This Work for New Zealand Horticulture. Involving New Zealand host plants in the development of the SPI for glassy-winged sharpshooter will benefit New Zealand agriculture, in the event that glassy-winged sharpshooter and/or *X. fastidiosa* are introduced there. The identification of invasion pathways for both glassy-winged sharpshooter and *X. fastidiosa*, as well as research to develop effective management tactics, must be given the highest priority in New Zealand, because of their potentially damaging effects on horticulture and ultimately New Zealand's national economy. Dispersal of *X. fastidiosa* in New Zealand could be aided by a favorable climate and ready availability of insect-favored host plants. Awareness of which potential glassy-winged sharpshooter host plants are acceptable for xylem ingestion, the impact of such feeding on *X. fastidiosa* transmission, and the role of other native and/or exotic fauna in transmission of the bacterium is paramount to minimize its impact in New Zealand, should glassy-winged sharpshooter carrying *X. fastidiosa* become established there. Although there is no record of *X. fastidiosa* present in New Zealand, the meadow spittle bug (*Philaenus spumarius*) is common in New Zealand and is a vector of *X. fastidiosa* (Redak et al. 2004). Although grape and citrus have been studied as hosts of glassy-winged sharpshooter, this is the first quantitative study that has found that apple is also a highly acceptable host. This study is also the first to show how glassy-winged sharpshooter changes its stylet penetration behaviors on pohutukawa, a less acceptable host than apple or grape. Although, ultimately, the same amount of ingestion occurred on the three uncontaminated host plants, large differences in preingestive, testing/acceptance behaviors were demonstrated on pohutukawa compared with apple or grape. Some of these testing behaviors also may represent the *X. fastidiosa* inoculation behavior, and thus reveal differences in susceptibility to inoculation by pohutukawa, and perhaps some other native New Zealand plants.

Even in the absence of *X. fastidiosa*, the feeding of glassy-winged sharpshooter by itself could cause significant damage to apple and other horticultural crops. An important aspect of the horticulture industry is New Zealand's clean and green image, and the value this adds to New Zealand produce. The apple export market in particular is likely to be economically damaged if fruit becomes covered with excreta during glassy-winged sharpshooter feeding, as has been common with citrus in California. Excreta dry to leave an unsightly residue that may not be economically possible to remove. Thus, the potential damage to New Zealand agriculture from glassy-winged sharpshooter, with or without *X. fastidiosa*, supports continued cooperation with California researchers to study glassy-winged sharpshooter feeding on an array of host plants.

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